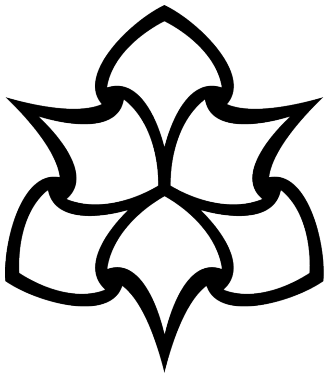

British Journal of Pharmacology

British Journal of Pharmacology (2019) **176** 988–999 988

Themed Section: Eicosanoids 35 years from the 1982 Nobel: where are we now?

REVIEW ARTICLE

Eicosanoids in platelets and the effect of their modulation by aspirin in the cardiovascular system (and beyond)

Correspondence Timothy D. Warner, Centre for Immunobiology, Blizard Institute, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, London E1 2AT, UK. E-mail: t.d.warner@qmul.ac.uk

Received 8 November 2017; **Revised** 21 February 2018; **Accepted** 22 February 2018

Marilena Crescente, Laura Menke, Melissa V Chan, Paul C Armstrong and Timothy D Warner

Centre for Immunobiology, Blizard Institute, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, London, UK

Platelets are important players in thrombosis and haemostasis with their function being modulated by mediators in the blood and the vascular wall. Among these, eicosanoids can both stimulate and inhibit platelet reactivity. Platelet Cyclooxygenase (COX)-1-generated Thromboxane (TX)A_2 is the primary prostanoid that stimulates platelet aggregation; its action is counter-balanced by prostacyclin, a product of vascular COX. Prostaglandin (PG)D_2, PGE_2 and 12-hydroxyeicosatraenoic acid (HETE), or 15-HETE, are other prostanoid modulators of platelet activity, but some also play a role in carcinogenesis. Aspirin permanently inhibits platelet COX-1, underlying its anti-thrombotic and anti-cancer action. While the use of aspirin as an anti-cancer drug is increasingly encouraged, its continued use in addition to P_2Y_{12} receptor antagonists for the treatment of cardiovascular diseases is currently debated. Aspirin not only suppresses TXA_2 but also prevents the synthesis of both known and unknown antiplatelet eicosanoid pathways, potentially lessening the efficacy of dual antiplatelet therapies.

LINKED ARTICLES

This article is part of a themed section on Eicosanoids 35 years from the 1982 Nobel: where are we now? To view the other articles in this section visit http://onlinelibrary.wiley.com/doi/10.1111/bph.v176.8/issuetoc

Abbreviations

AA, arachidonic acid; CYP450, cytochrome P450; EETs, epoxyeicosatrienoic acids; ECs, endothelial cells; HETE, hydroxyeicosatraenoic acid; LOX, lipoxygenase; NSAIDs, nonsteroidal anti-inflammatory drugs; PGI_2, prostacyclin; PUFAs, polyunsaturated fatty acids; USPSTF, US Preventive Services Task Force

DOI:10.1111/bph.14196

Introduction

Platelets play a fundamental role in maintaining haemostasis. A fine balance exists in which platelets can be rapidly activated to aggregate and form a plug that prevents bleeding. But when platelets get inappropriately activated, thrombi form within the vessel wall which can lead to thrombotic events such as heart attack and stroke. The activation or inhibition of platelets can be modulated by many agents with a central role being played by eicosanoids. **TXA_2** and prostacyclin (**PGI_2**) are the main eicosanoids affecting the function of platelets. The groups of Vane and Samuelsson were pioneers in their identification and in establishing their action on platelets and on the vasculature (Bunting *et al.*, 1977; Bunting *et al.*, 1983; Moncada *et al.*, 1976; Moncada *et al.*, 1978; Needleman *et al.*, 1976; Svensson *et al.*, 1975; Whittaker *et al.*, 1976).

Since their discovery, and with the continued development of analytical techniques such as mass spectrometry-based lipidomics, hundreds of structurally and stereochemically distinct eicosanoid families have been identified (Harkewicz and Dennis, 2011).

This review will focus on the production of eicosanoids by platelets and endothelium and their effect on platelet function in the cardiovascular system. We will discuss how **aspirin** modulates the synthesis of these eicosanoids and the consequences on its anti-thrombotic efficacy. Laboratory techniques to evaluate response to aspirin will be also presented, and their ability to predict the occurrence of cardiovascular events will be examined. Finally, recent advances in understanding the role of platelet-related eicosanoids in cancer will be presented.

Eicosanoids and the fine regulation of platelet function and haemostasis

Eicosanoids are mainly derived from **arachidonic acid (AA)** but can also be generated from other 20 carbon polyunsaturated fatty acids (PUFAs), such as dihomo-γ-linolenic acid, an ω-6-derived PUFA, or eicosapentaenoic acid (Subhash *et al.*, 2007). These fatty acids are released from the cellular phospholipid membrane *via* the action of the enzyme **phospholipase A_2 (PLA_2)** and subsequently converted *via* the COXs into TXA_2 and PGs, such as PGI_2, **PGE_2** and **PGD_2**, *via* **lipoxygenases (LOXs)** into hydroxyeicosatraenoic acids (e.g. **12-HETE**), and *via* cytochrome P450 (**CYP450**) enzymes into epoxyeicosatrienoic acids (**EETs**) (Dennis and Norris, 2015).

Platelets can produce significant amounts of TXA_2, PGE_2, PGD_2, 11-, 12- and 15-HETE dependent upon the activity of **cytosolic group IV A PLA_2**, a widely expressed PLA_2 isoform (Kirkby *et al.*, 2015; Rauzi *et al.*, 2016). Below, we will discuss platelet and non-platelet-derived eicosanoids whose actions modulate platelet function and consequentially haemostasis and thrombosis (Figure 1).

COX-dependent eicosanoids

COX, more precisely known as PGH synthase, converts AA first into **PGG_2**, *via* a COX function and then to **PGH_2** following a peroxidase reaction (Smith and Dewitt, 1996). PGH_2 is an unstable molecule and, in platelets, undergoes further transformations catalysed by TX synthase, PGD isomerase or PGE synthase to form TXA_2, PGD_2 or PGE_2 respectively.

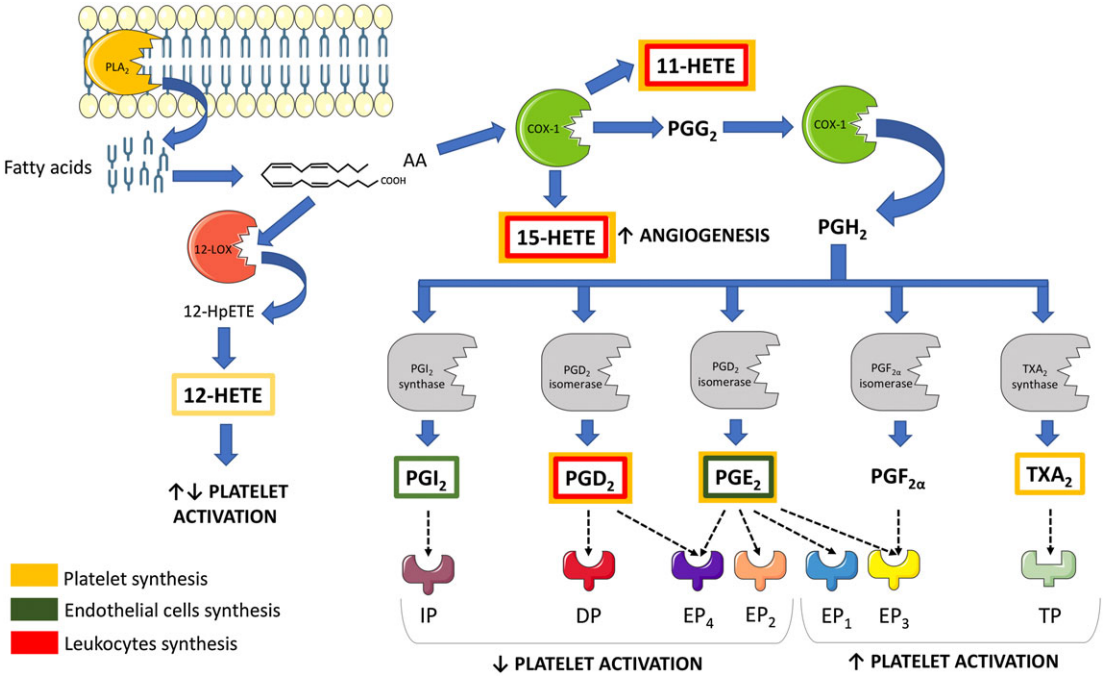

Figure 1

Diagram of the biosynthesis of the main eicosanoids that affect platelet function and where it occurs. The yellow, green and red boxes represent the origin of the eicosanoids as platelets, ECs and leukocytes respectively. The receptors for each eicosanoid are shown as well as the associated effects on platelet activation.

Two different isoforms of COX exist in the cardiovascular system, namely, **COX-1** and **COX-2** (Hla and Neilson, 1992; Kujubu *et al.,* 1991; Masferrer *et al.,* 1992; O'Banion *et al.,* 1992; Xie *et al.,* 1991). COX-1 is usually considered a constitutive form (Kirkby *et al.,* 2012; Langenbach *et al.,* 1997), while COX-2 is considered to be an inducible enzyme, although a role for constitutive COX-2 has been shown in the kidneys and the central nervous system (Herschman *et al.,* 1997; Mitchell and Warner, 2006). Platelets mainly express COX-1, but traces of COX-2 have been detected, possibly carried over from megakaryocytes, the platelet precursor cells, or as a result of the transcription of residual mRNA into protein (Rocca *et al.,* 2002; Warner *et al.,* 2011).

Thromboxane A_2

The most directly important prostanoid for platelet function is COX-1-generated TXA_2. It was first identified by Vane as a 'rabbit-aorta-contracting substance' (RCS) produced by the lungs during anaphylaxis (Piper and Vane, 1969). Later, TXA_2 was shown to be synthesized by activated platelets and to act in an autocrine and paracrine manner to induce thrombosis (Smith and Willis, 1971). On platelets, TXA_2 binds to the thromboxane **prostanoid (TP**) receptor and initiates an amplification loop leading to further platelet activation, aggregation and TXA_2 formation (Reilly and Fitzgerald, 1993). The TP receptor can couple with several G proteins, such as $G_{12/13}$, leading to platelet shape change *via* phosphorylation of the myosin light chain, platelet granule release and irreversible aggregation (Smyth, 2010). In the vasculature, TXA_2 induces vasoconstriction and the proliferation of vascular smooth muscle cells.

PGI_2 (prostacyclin)

When first discovered as an autacoid produced by vascular tissue, PGI_2 or prostacyclin was named as PGX and was described as a substance which, in contrast to TXA_2, inhibited the clumping of platelets and relaxed vascular strips (Moncada *et al.,* 1976). Now known to be predominantly produced by the endothelium within blood vessels, there has been strong debate as to which isoform of COX catalyses the vascular production of PGI_2. Although still controversial, research by ourselves and colleagues strongly suggests that, in the healthy vasculature, PGI_2 production is driven by COX-1 (Bolego *et al.,* 2009; Evangelista *et al.,* 2006; Kirkby *et al.,* 2012; Yu *et al.,* 2012). This is discussed in more detail elsewhere in this issue (Mitchell and Kirkby, 2018).

Endothelium-produced PGI_2 binds to the **G_s-coupled PGI_2 receptor (IP**) on platelets and generally reduces platelet reactivity, which can be critical to minimizing the risk for atherothrombotic events (Midgett *et al.,* 2011). Binding of PGI_2 to the IP receptor results in the activation of **adenylate cyclase** and a subsequent rise in **cAMP** levels in platelets (Yang *et al.,* 2002). This stimulates phosphorylation of **PKA**, which suppresses various signalling pathways involved in platelet function such as adhesion, aggregation and granule secretion. With regard to the subject of this review, PKA activation decreases the release of Ca^{2+} from internal stores, reducing the activation of cytosolic PLA2 ($cPLA_2$) and the liberation of AA from the phospholipid membrane, and so diminishing the production of platelet-derived eicosanoids, such as TXA_2 (den Dekker *et al.,* 2002).

PGD_2

PGD_2 is well established as a macrophage product but, in lesser amounts, is also synthesized by platelets. By interaction with platelet **DP_1 receptors**, PGD_2 increases adenylyl cyclase activity and so, like PGI_2, inhibits platelet activation (Bushfield *et al.,* 1985; Oelz *et al.,* 1977; Whittle *et al.,* 1978).

PGE_2

PGE_2 is released by endothelial cells (ECs) and, to some extent, by activated platelets. It acts on a range of prostanoid receptors, **EP_1** - **EP_4**, that differently modulate second messengers, such as cAMP and free Ca^{2+}, within platelets and exert contrasting effects on platelet function (Deeb *et al.,* 2008; Yang *et al.,* 2002). The effects on platelets of PGE_2 acting through EP receptors are concentration dependent. At low concentrations (0.1–10 $\mu mol \cdot L^{-1}$), PGE_2 binds to G_i-coupled receptors (**EP_3**) to enhance aggregation, whereas at higher concentrations (100 $\mu mol \cdot L^{-1}$), it activates G_s-coupled receptors (**EP_2**, EP_4) to inhibit aggregation (Friedman *et al.,* 2015; Glenn *et al.,* 2012; Petrucci *et al.,* 2011). Stimulation of EP_3 receptors by PGE_2 decreases cAMP levels, thus favouring platelet aggregation, but the full effect is only seen in the presence of another platelet agonist (Fabre *et al.,* 2001; Friedman *et al.,* 2015). On the other hand, the increased cAMP levels which accompany EP_4 receptor activation correlate with suppressed platelet aggregation (Glenn *et al.,* 2012).

In addition to PGE_2, **PGE_1**, **$PGF_{2\alpha}$** and PGD_2 can also bind to EP_3 and EP_4 receptors but with lower affinity and reversible effects (Armstrong *et al.,* 1985; Friedman *et al.,* 2015; Glenn *et al.,* 2012).

As well as the well-characterized effects of PGE_2 mediated through EP_3 and EP_4 receptors, EP_1 receptors are also expressed on platelets (Kauskot and Hoylaerts, 2012; Petrucci *et al.,* 2011). Although the signal transduction pathway is not clear, studies in several cell lines expressing EP_1 receptors suggest that its activation increases Ca^{2+} influx and might thereby stimulate platelet aggregation (Whittle *et al.,* 2012).

While PGE_2 seems to both inhibit and potentiate platelet aggregation *in vitro*, a study by Gross *et al.* has elegantly shown that, *in vivo,* PGE_2 is produced by the vessel wall or after the rupture of a plaque. Under these conditions, PGE_2 activates the EP_3 receptors on platelets and clearly enhances, rather than reduces, thrombus formation in the arterial vessel wall (Gross *et al.,* 2007).

LOX-dependent 12-HETE

12-HETE is the major **12-LOX**-catalysed metabolite and the most abundant eicosanoid produced by platelets upon stimulation (Kirkby *et al.,* 2015; Rauzi *et al.,* 2016), but its effects on platelet function are not completely understood. Initial studies suggested that both 12-HETE and 14-hydroxy-**docosahexaenoic acid** (14-OH-DHA), the 12-LOX-derived metabolite of DHA, inhibit platelet aggregation initiated by the TP receptor agonist **U46619** (Croset *et al.,* 1988). In agreement with these data, platelet-specific knockout of 12-LOX in mice resulted in hypersensitivity to **ADP**-induced aggregation, which was reversed by incubation with exogenous 12-HETE. However, lack of 12-LOX did not affect collagen-induced aggregation or platelet adhesion

(Johnson *et al.,* 1998). Interestingly, another study reported that inhibition of 12-LOX led to decreased platelet aggregation that correlated with a significant reduction of 12-HETE in response to collagen (Maskrey *et al.,* 2014). A recent review concluded that 12-HETE can exert both pro- and anti-aggregatory effects on platelets that depend crucially on 12-HETE concentration, stereospecificity and co-incubation with different agonists (Porro *et al.,* 2014). Platelets also produce hepoxilins from the precursor **12-hydroperoxyeicosatetraenoic acid**. Hepoxilin has shown to exert anti-thrombotic effects in platelets (Margalit *et al.,* 1995), most likely *via* inhibition of TXA_2 formation and blockade of the TP receptor (Reynaud, 2002).

Platelet-cellular crosstalk and eicosanoid biosynthesis

Transcellular routes through which platelets exchange eicosanoids with ECs or leukocytes are important to vascular homeostasis as well as to processes such as vascular inflammation. Some of these cellular crosstalk pathways are depicted in Figure 2 and discussed below. For example, ECs can utilize PGH_2 released from platelets to produce PGI_2. This suggests a counteractive mechanism in which activated platelets that are in direct contact with the vessel wall produce endoperoxide that can in turn be used by ECs to inhibit platelet functions and stimulate the return to homeostasis (Marcus *et al.,* 1980; Porro *et al.,* 2014).

CYP450 epoxygenases can convert AA into the biologically active EETs. The main producers of EETs are vascular ECs which not only release EETs following stimulation and contribute to vasodilation but also promote anti-inflammatory effect in the vascular system (Yang, 2015). EETs also have potent anti-adhesive and anti-aggregatory activities which they exert by causing hyperpolarization of the platelet membrane (Sudhahar *et al.,* 2010).

In the cardiovascular system, leukocytes represent the main source of **5-LOX**-derived LTs. These metabolites potentiate **adrenaline** and **thrombin**-induced platelet aggregation, probably by increasing the activity of TXA_2 synthetase and thereby TXA_2 formation (Mehta *et al.,* 1986). On the other hand, platelets can utilize leukocyte-derived **LTA_4** as a precursor for lipoxin production. Following release, **lipoxin A_4** acts on platelets *via* the **FPR2/ALX receptor** (Czapiga *et al.,* 2005) and mediates protective functions by suppressing platelet adhesion, TXA_2 formation and platelet–neutrophil interaction (Ortiz-Muñoz *et al.,* 2014). With regard to inflammation, platelets can transfer eicosanoid precursors to leukocytes which are fundamental for the formation of pro-resolving mediators. A prominent example is the epoxy-resolvins, which are produced by platelet 12-

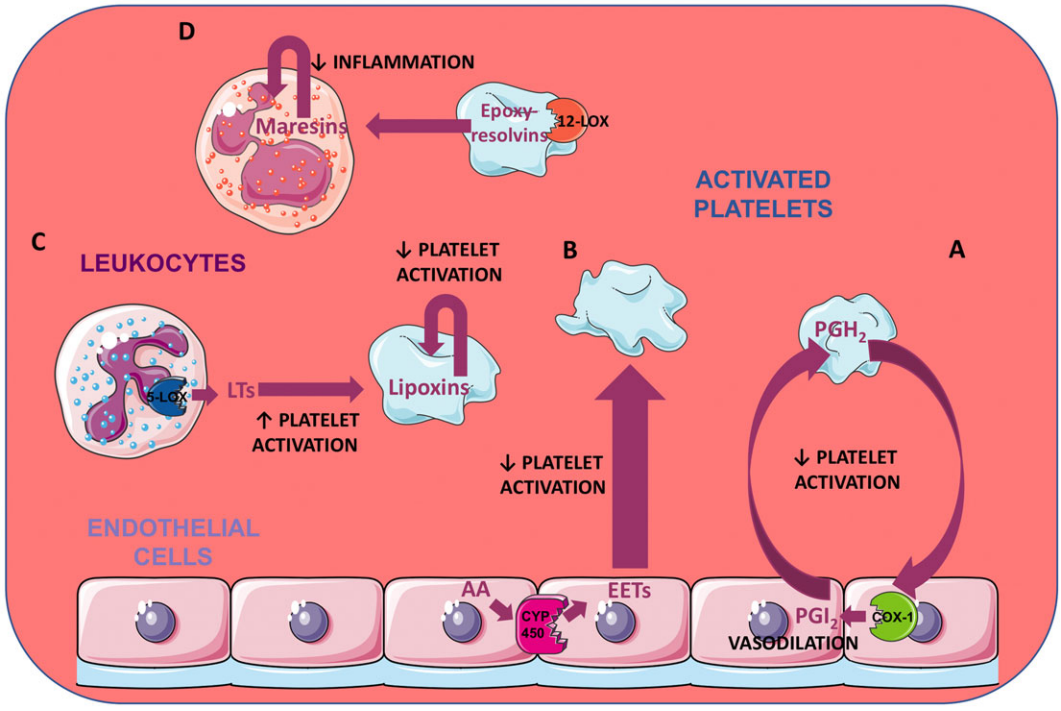

Figure 2

Main pathways of eicosanoid-mediated crosstalk between platelets and other cells. The eicosanoid exchanges between platelets and ECs and their effects on the vessel homeostasis are illustrated in (A) and (B). Some of the PGH_2 released by platelets may be used by COX-1 in the ECs to produce PGI_2 which induces vasodilation and prevents further platelet activation (A). ECs, on the other hand, can synthesize EETs starting from AA, through the action of CYP450. EETs reduce platelet activation (B). (C) and (D) represent some routes of platelet-leukocyte crosstalk. LTs are synthesized in leukocytes by 5-LOX and act together with other agonists to potentiate platelet activation. However, platelets can also use LTs to make lipoxins which reduce the activation of platelets (C). 12-LOX in platelets also produces epoxy-resolvins that can be used by the leukocytes to make maresins, molecules important for the resolution of inflammation (D).

LOX and transferred to neutrophils where they are transformed into maresins, which are molecules with important roles in terminating acute inflammatory responses (Abdulnour *et al.,* 2014).

Modulation of eicosanoid production by platelets and the anti-thrombotic efficacy of aspirin

John Vane reported for the first time that aspirin inhibits the production of PGs (Vane, 1971). This mechanism was identified as the basis of the therapeutic action of nonsteroidal anti-inflammatory drugs (NSAIDs) (Vane, 1971) and was confirmed in platelets by Smith and Willis (1971). Many NSAIDs have been developed since then, and we know now that these compounds affect eicosanoid biosynthesis through the inhibition of both COX-1 and COX-2. COX-1 and COX-2 are expressed to differing levels in different tissues and under different conditions of health and disease. Such differences and their significance has been reviewed extensively (Khan *et al.,* 2002; Mitchell and Warner, 2006; Wallace and Devchand, 2005).

In the context of platelet function, only aspirin produces irreversible inhibition of COX-1 through its ability to covalently modify the enzyme (Cerletti *et al.,* 1982; Loll *et al.,* 1995). Consequently, aspirin impairs the synthesis of TXA_2 for the entire platelet lifespan, and this explains its general antithrombotic action (Ferreira *et al.,* 1971; Smith and Willis, 1971; Vane, 1971), although under some circumstances aspirin-treated platelets may be able to recover the ability to synthesize TXA_2 after *de novo* synthesis of COX-1 (Evangelista *et al.,* 2006). Because of its irreversible action, the antiplatelet effects of aspirin are seen with low doses of 50–100 $mg{\cdot}day^{-1}$ (Patrignani *et al.,* 1982; Patrono, 2005; Warner *et al.,* 2011). Aspirin is commonly given in combination with antagonists of ADP, acting at **P_2Y_{12} receptor**, such as **clopidogrel**, **prasugrel** or **ticagrelor** (Bhatt, 2009; Gargiulo *et al.,* 2016; Investigators TCIUaTPRET, 2001; Patrono *et al.,* 2011; Wallentin *et al.,* 2009; Windecker *et al.,* 2014; Wiviott *et al.,* 2007). Despite the proven anti-thrombotic efficacy of this dual therapy, many studies are currently investigating the benefits of single antiplatelet-drug therapy, using newer drugs such as ticagrelor (Gargiulo *et al.,* 2016). The hope is to retain the anti-thrombotic effects of dual antiplatelet therapy while lessening the unwanted side effects. This rationale is not only based on the need to reduce the bleeding risk associated with the dual antiplatelet therapy (Du *et al.,* 2016; Maree and Fitzgerald, 2007) but also because evidence suggests that P_2Y_{12} antagonists alone can decrease platelet TXA_2 production and reduce aggregation mediated by TP receptor activation (Armstrong *et al.,* 2010; Armstrong *et al.,* 2011; Bhavaraju *et al.,* 2010; Kirkby *et al.,* 2011). Furthermore, the ability of aspirin to reduce the production of vascular PGI_2 directly by inhibiting COX-1 in ECs or indirectly by inhibiting COX-1 in other cells supplying precursors of PGI_2, such as PGH_2, could produce a pro-thrombotic effect that reduces the overall efficacy of dual antiplatelet therapy (Björkman *et al.,* 2013; FitzGerald *et al.,* 1983; Franchi *et al.,* 2016; Mahaffey *et al.,* 2011; Maree and Fitzgerald, 2007; Warner *et al.,* 2010; Warner *et al.,* 2016). Therefore, it is necessary not only to seek therapeutic strategies apart from aspirin, but also to extensively re-evaluate the effects of aspirin *in vivo*. This last goal could be achieved by using more recently developed techniques such as liquid chromatography–tandem mass spectrometry or the genetic manipulation of animals. For example, we have recently found, through the use of mass spectrometry analysis, that aspirin prevents not only the synthesis of TXA_2 by platelets but also the production of PGD_2, PGE_2, 11-HETE and **15-HETE**. PGD_2 and PGE_2 are PGs with antiplatelet actions and their inhibition can further contribute to a reduced efficacy of the antithrombotic treatments (Rauzi *et al.,* 2016). In addition, our own recently developed animal models where the expression of COX-1 is specifically ablated in ECs or in megakaryocytes/platelets will be useful in dissecting the effects of eicosanoids on the cardiovascular system and the outcomes of aspirin treatment.

Eicosanoid measurements and platelet function tests to evaluate the efficacy of aspirin in cardiovascular patients

The way platelets respond to treatment with aspirin can be monitored in the laboratory either by techniques that specifically measure platelet COX-1 activity or by tests assessing other platelet activation pathways besides COX-1.

The measurement of platelet-generated eicosanoids, in particular of TXB_2, the stable form of TXA_2, either in serum or after *in vitro* stimulation of platelets, falls in the first category of techniques. With a strong stimulus, the levels of TXB_2 can be taken as reflecting the maximal capacity of platelets to synthesize TXA_2 *via* the COX-1 pathway and this can be regarded as a sensitive measure of the response to aspirin, in the laboratory (Cattaneo, 2007; Maree and Fitzgerald, 2007; Ohmori *et al.,* 2006). On the other hand, the levels of the main TXA_2 metabolite found in urine, **11- dehydro TXB_2**, reflect systemic TXA_2 generation and may not only reflect the effect of aspirin on platelet COX-1 (Kirkby *et al.,* 2012; Kirkby *et al.,* 2015; Smith *et al.,* 2012).

Another standard test for studies of platelet inhibition by aspirin is light transmission aggregometry, which measures the ability of platelets to aggregate after being stimulated. Different stimuli can be used in this test to explore different aspects of platelet activation. AA is a substrate for COX-1, so the aggregation response to this agonist closely reflects platelet COX-1 activity, while ADP or collagen induces platelet aggregation through pathways that are not exclusively dependent on COX-1 activation (Thiagarjan and Wu, 2002). Other methodologies, such as flow cytometry evaluation of markers of platelet activation and secretion or of the formation of platelet-leukocyte aggregates, can also be used to assess platelet inhibition by aspirin. Moreover, semi-automated point-of-care platelet function assays, such as the PFA-100® system and RPFA-Verify-Now Aspirin, have been introduced (Frelinger *et al.,* 2006).

The prevalence of aspirin resistance, that is, lack of effect of aspirin, reported in the literature is largely based on various

non-specific laboratory techniques and, in general, aspirin resistance is much lower when measured with COX-1 specific methods (Gurbel *et al.,* 2007; Lordkipanidzé *et al.,* 2007).

It is generally held that aspirin should inhibit platelet TXA_2 synthesis by at least 95% to reach a functional effect, and this assumption is mainly based on the observation that there is a non-linear relationship between inhibition of platelet TXA_2 synthesis and inhibition of platelet aggregation (Kidson-Gerber *et al.,* 2010; Santilli *et al.,* 2009). However, due to the technical limitations of the tests employed, platelet response to aspirin is usually evaluated using one or two agonists, often at fixed concentration that does not make it possible to properly characterize biological variations in drug response. Recently, we have developed a test using optical multichannel platelet aggregometry in a 96-well-plate, that can explore platelet function in response to a broad range of agonists and agonist concentrations (Chan *et al.,* 2011; Lordkipanidzé *et al.,* 2014). This test has indicated that there is a linear relationship between TXA_2 synthesis and TXA_2-mediated platelet aggregation, in the presence of different levels of COX-1 inhibition and could represent a valid alternative method of reliably identifying responders to treatment with aspirin (Armstrong *et al.,* 2008).

The association between a high platelet reactivity while on treatment, and the risk of patients having a thrombotic event is uncertain (Consuegra-Sánchez *et al.,* 2013; Depta *et al.,* 2012; Li *et al.,* 2014; Tantry *et al.,* 2013). However, four different meta-analyses have so far indicated that the lack of response to aspirin, as detected in the laboratory, may predict clinical recurrences (Crescente *et al.,* 2008a; Crescente *et al.,* 2008b; Krasopoulos *et al.,* 2008; Reny *et al.,* 2008; Snoep *et al.,* 2007). It also appears, from some of the studies performed in this area, that a combination of tests and of different agonists is better than one single test to establish this type of association (Armstrong *et al.,* 2008; Crescente *et al.,* 2011; Gremmel *et al.,* 2015; Smith *et al.,* 2012) and a summary of these observations is provided in Figure 3**.** However, it is essential that additional biomarkers of response to aspirin are identified and larger epidemiological studies performed, before any change of an antiplatelet treatment is made on the basis of laboratory test results. Notably, there have been no clinical trials demonstrating that tailoring antiplatelet therapy to results from *ex vivo* platelet testing, produces an improvement in patient outcomes (Collet *et al.,* 2012; Depta *et al.,* 2012).

Anti-cancer effect of aspirin: role for platelet eicosanoids

In 1988, Kune *et al.* reported for the first time an association between the intake of aspirin and a reduced risk of colorectal cancer, thus extending the therapeutic potential of aspirin beyond its use as an anti-inflammatory or anti-thrombotic drug. This observation was confirmed by many subsequent epidemiological studies and by a large meta-analysis which also showed that aspirin reduced the risk of gastrointestinal cancers in general (Algra and Rothwell, 2012; Burn *et al.,* 2008; Burn *et al.,* 2011; Cole *et al.,* 2009; Cuzick *et al.,* 2015; Rothwell *et al.,* 2012). As well as aspirin, non-aspirin NSAIDS and, in particular, COX-2 selective inhibitors, such as **celecoxib** and **rofecoxib**, were widely reported to prevent colonic tumourigenesis (Arber *et al.,* 2006; Arber *et al.,* 2011; Baron *et al.,* 2006; Bertagnolli *et al.,* 2006; Cao *et al.,* 2016; Steinbach *et al.,* 2000). However, concerns about the prothrombotic effects of non-aspirin NSAIDs including COX-2 inhibitors (Baron *et al.,* 2006; Baron *et al.,* 2008; Collaboration CaTNTC, 2013) have ended cancer prevention trials

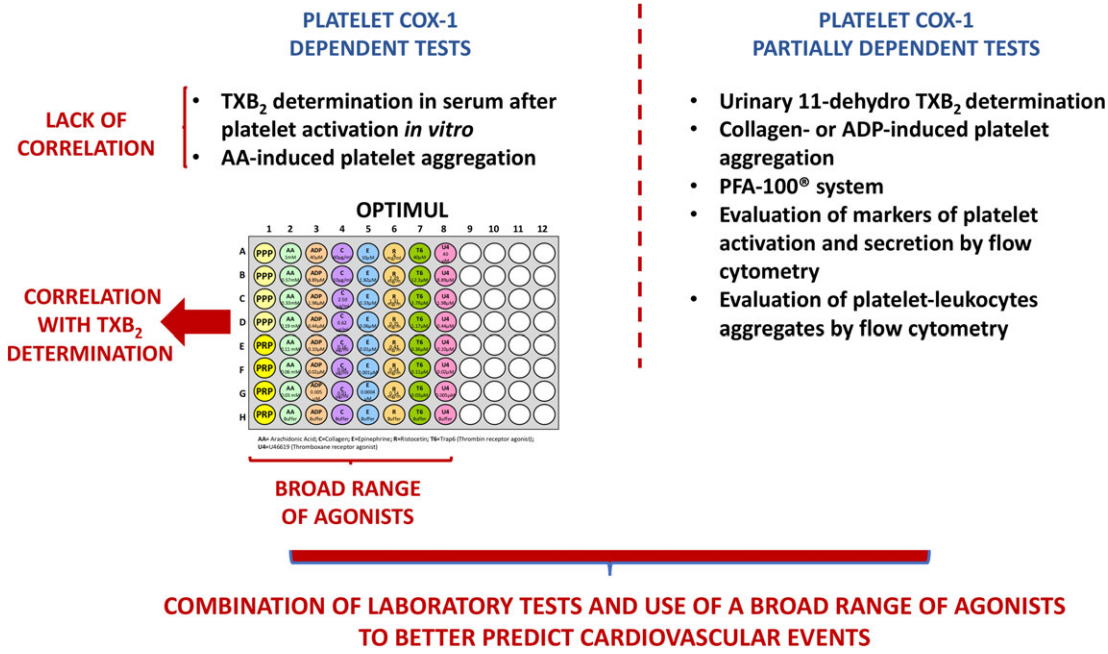

Figure 3
Schematic representation of platelet function tests used to monitor responses to aspirin in cardiovascular patients.

using COX-2 inhibitors , and the US Preventive Services Task Force (USPSTF) no longer supports the use of non-aspirin NSAIDs for the prevention of colorectal cancer.

In contrast, aspirin is the only drug with no cardiovascular risk that is effective in both primary and secondary prevention of colorectal cancer and also reduces the incidence and risk of all-cause cancer mortality (Cuzick *et al.,* 2015; Rothwell *et al.,* 2011). As aspirin is used in prevention of cardiovascular diseases and the most colorectal cancer cases are diagnosed after the age of 50, the last guidelines from the USPSTF recommend low-dose aspirin for the primary prevention of colorectal cancer in patients at increased cardiovascular risk (Bibbins-Domingo, 2016).

The follow-up studies of many clinical trials indicate that the chemoprotective action of aspirin can be detected at a dose as low as 75 mg·day^{-1}. Furthermore, it is saturable at these low doses and is present when using a controlled-release aspirin formulation that mainly targets platelet COX-1 (Patrignani and Patrono, 2016). These findings have been confirmed by studies showing that small doses of aspirin, by blocking the formation of platelet TXA_2, PGE_2, PG-containing oxidized phospholipids and **sphingosine 1-phosphate**, reduce the exchange of lipid mediators between platelets and cancer cells in the tumour microenvironment (Aldrovandi *et al.,* 2013; Dovizio *et al.,* 2013; Ulrych *et al.,* 2011).

Strong evidence also suggests that eicosanoids linked to COX-1 activity act as pro-angiogenic factors and therefore the anti-cancer effects of aspirin are also related to a reduction of angiogenesis (Etulain *et al.,* 2013; Rauzi *et al.,* 2016). For example, we have recently found that platelet COX-1-derived 15(S)-HETE induces an angiogenic response in HMEC-1 cells and rat aortic rings and this effect disappears in presence of aspirin, when the synthesis of 15(S)-HETE is blocked (Rauzi *et al.,* 2016). In addition to the eicosanoids, platelets can release a variety of pro-angiogenic factors from their α-granules and this release can be modulated by treatment with aspirin, as well (Coppinger *et al.,* 2004).

Platelets promote cancer progression also by favouring the metastatic process. In particular, platelets will form aggregates around tumour cells in the bloodstream, that protect tumor cells from being cleared by the immune system (Gay and Felding-Habermann, 2011). Also, when COX-1 activity is blocked by aspirin or when a PGE_2 antagonist is used, platelets lose the ability to transform human colon carcinoma cells into mesenchymal-like cancer cells. Moreover, the administration of aspirin to mice prevents the platelet-induced formation of metastases in the lungs, and this is associated with a reduced systemic synthesis of TXA_2 and PGE_2 (Guillem-Llobat *et al.,* 2016).

This evidence suggests that the anti-cancer efficacy of aspirin resides in its ability to block the biosynthesis of platelet-derived eicosanoids, which not only serve as substrates for other cells present in the tumour microenvironment but also promote angiogenesis and the metastatic progression of the tumour (Figure 4). While there is strong evidence for aspirin having beneficial effects in gastrointestinal cancers, the efficacy of aspirin in other cancer types such

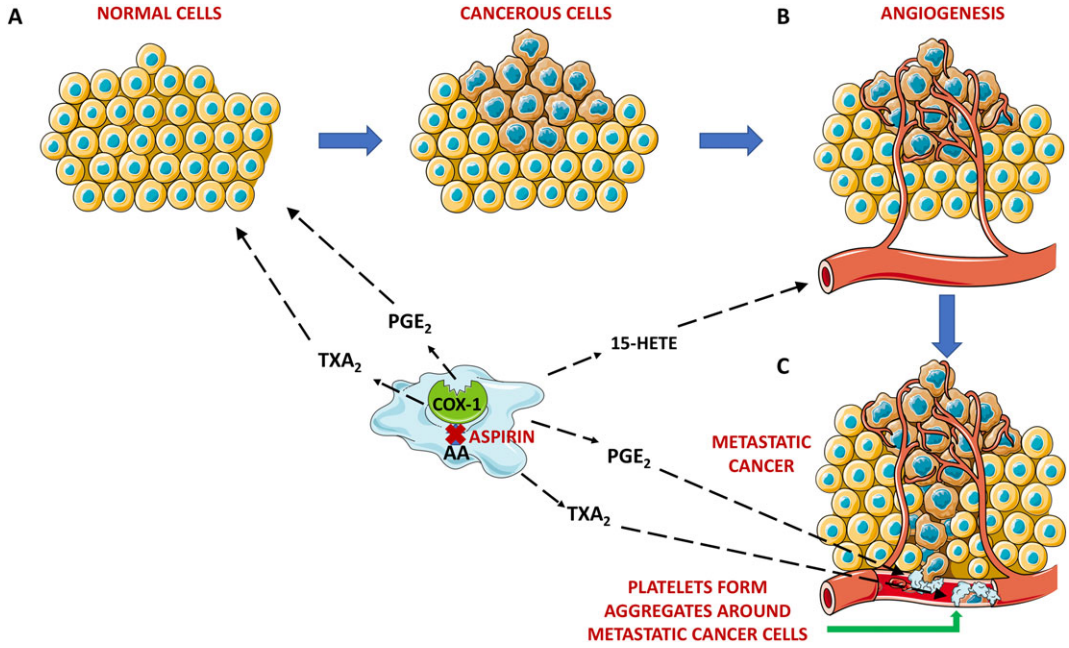

Figure 4

Effects of platelet COX-1-derived eicosanoids and of aspirin treatment in the progression of cancer. The preventive role of aspirin in the progression of cancer depends at least in part on its ability to block the formation of eicosanoids by platelet COX-1. TXA_2 and PGE_2 are released in the tumour micro-environment and favour the transformation of cells from a normal to a cancerous phenotype (A). 15-HETE is another eicosanoid synthesised by COX-1 in platelets that promotes angiogenesis, a process that further promotes cancer progression (B). TXA_2 and PGE_2 mediate the formation of platelet aggregates around the metastatic cancer cells, protecting them from the immune system and assisting their spread throughout the body (C).

as gastroesophageal, breast and prostate cancers has still to be evaluated, as well as the most appropriate timings and doses that can be used to maximize its anti-carcinogenic effects (Patrignani and Patrono, 2016).

Conclusions

Eicosanoids produced by platelets, or made from other cells, are important modulators of platelet function and regulate the fine balance between haemostasis and thrombotic disease. The eicosanoid-mediated crosstalk between platelets and other cells also regulates pathophysiological processes such as cancer. Low doses of aspirin, through their ability to inhibit platelet COX-1 and the synthesis of pro-aggregatory TXA_2, is still nowadays considered as a first choice treatment to reduce the risk of thrombotic events. Ongoing research may lead to the replacement of aspirin in this role by $P2Y_{12}$ receptor antagonists, while aspirin continues to be used for protection against the development of a range of cancers.

Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Harding *et al.,* 2018), and are permanently archived in the Concise Guide to PHARMACOLOGY 2017/18 (Alexander *et al.,* 2017a,b).

Acknowledgements

All the figures were produced using Servier Medical Art (http://www.servier.com). This work was supported by grants from the British Heart Foundation (PG/15/47/31591; PG/15/79/31777 and PG/17/40/33028) and European Commission (H2020-MSCA-ITN-2015 GA 675111) to T.D.W.

Conflict of interest

The authors declare no conflicts of interest.

References

Abdulnour R-EE, Dalli J, Colby JK, Krishnamoorthy N, Timmons JY, Tan SH *et al.* (2014). Maresin 1 biosynthesis during platelet–neutrophil interactions is organ-protective. Proc Natl Acad Sci U S A 111: 16526–16531.

Aldrovandi M, Hammond VJ, Podmore H, Hornshaw M, Clark SR, Marnett LJ *et al.* (2013). Human platelets generate phospholipid-esterified prostaglandins via cyclooxygenase-1 that are inhibited by low dose aspirin supplementation. J Lipid Res 54: 3085–3097.

Alexander SPH, Christopoulos A, Davenport AP, Kelly E, Marrion NV, Peters JA *et al.* (2017a). The Concise Guide to PHARMACOLOGY 2017/18: G protein-coupled receptors. Br J Pharmacol 174 (Suppl 1): S17–S129.

Alexander SPH, Fabbro D, Kelly E, Marrion NV, Peters JA, Faccenda E *et al.* (2017b). The Concise Guide to PHARMACOLOGY 2017/18: Enzymes. Br J Pharmacol 174 (Suppl 1): S272–S359.

Algra AM, Rothwell PM (2012). Effects of regular aspirin on long-term cancer incidence and metastasis: a systematic comparison of evidence from observational studies versus randomised trials. Lancet Oncol 13: 518–527.

Arber N, Eagle CJ, Spicak J, Rácz I, Dite P, Hajer J *et al.* (2006). Celecoxib for the prevention of colorectal adenomatous polyps. N Engl J Med 355: 885–895.

Arber N, Spicak J, Racz I, Zavoral M, Breazna A, Gerletti P *et al.* (2011). Five-year analysis of the prevention of colorectal sporadic adenomatous polyps trial. Am J Gastroenterol 106: 1135–1146.

Armstrong PC, Dhanji AR, Tucker AT, Mitchell JA, Warner TD (2010). Reduction of platelet thromboxane A2 production ex vivo and in vivo by clopidogrel therapy. J Thromb Haemost 8: 613–615.

Armstrong PC, Leadbeater PD, Chan MV, Kirkby NS, Jakubowski JA, Mitchell JA *et al.* (2011). In the presence of strong P2Y12 receptor blockade, aspirin provides little additional inhibition of platelet aggregation. J Thromb Haemost 9: 552–561.

Armstrong PCJ, Truss NJ, Ali FY, Dhanji AA, Vojnovic I, Zain ZNM *et al.* (2008). Aspirin and the in vitro linear relationship between thromboxane A2-mediated platelet aggregation and platelet production of thromboxane A2. J Thromb Haemost 6: 1933–1943.

Armstrong RA, Jones RL, Wilson NH (1985). Mechanism of the inhibition of platelet aggregation produced by prostaglandin F2 alpha. Prostaglandins 29: 601–610.

Baron JA, Sandler RS, Bresalier RS, Lanas A, Morton DG, Riddell R *et al.* (2008). Cardiovascular events associated with rofecoxib: final analysis of the APPROVe trial. The Lancet 372: 1756–1764.

Baron JA, Sandler RS, Bresalier RS, Quan H, Riddell R, Lanas A *et al.* (2006). A randomized trial of rofecoxib for the chemoprevention of colorectal adenomas. Gastroenterology 131: 1674–1682.

Bertagnolli MM, Eagle CJ, Zauber AG, Redston M, Solomon SD, Kim K *et al.* (2006). Celecoxib for the prevention of sporadic colorectal adenomas. N Engl J Med 355: 873–884.

Bhatt DL (2009). Role of antiplatelet therapy across the spectrum of patients with coronary artery disease. Am J Cardiol 103: 11A–19A.

Bhavaraju K, Georgakis A, Jin J, Gartner TK, Tomiyama Y, Nurden A *et al.* (2010). Antagonism of P2Y(1)(2) reduces physiological thromboxane levels. Platelets 21: 604–609.

Bibbins-Domingo K, USPST (2016). Aspirin use for the primary prevention of cardiovascular disease and colorectal cancer: U.S. preventive services task force recommendation statement. Ann Intern Med 164: 836–845.

Björkman J-A, Zachrisson H, Forsberg G-B, Von Bahr H, Hansson GI, Warner TD *et al.* (2013). High-dose aspirin in dogs increases vascular resistance with limited additional anti-platelet effect when combined with potent P2Y12 inhibition. Thromb Res 131: 313–319.

Bolego C, Buccellati C, Prada A, Gaion RM, Folco G, Sala A (2009). Critical role of COX-1 in prostacyclin production by human endothelial cells under modification of hydroperoxide tone. FASEB J 23: 605–612.

Bunting S, Moncada S, Vane JR (1977). Antithrombotic properties of vascular endothelium. Lancet 2: 1075–1076.

Bunting S, Moncada S, Vane JR (1983). The prostacyclin–thromboxane A2 balance: pathophysiological and therapeutic implications. Br Med Bull 39: 271–276.

Burn J, Bishop DT, Mecklin J-P, Macrae F, Möslein G, Olschwang S *et al*. (2008). Effect of aspirin or resistant starch on colorectal neoplasia in the lynch syndrome. N Engl J Med 359: 2567–2578.

Burn J, Gerdes A-M, Macrae F, Mecklin J-P, Moeslein G, Olschwang S *et al*. (2011). Long-term effect of aspirin on cancer risk in carriers of hereditary colorectal cancer: an analysis from the CAPP2 randomised controlled trial. The Lancet 378: 2081–2087.

Bushfield M, Mcnicol A, Macintyre DE (1985). Inhibition of platelet-activating-factor-induced human platelet activation by prostaglandin D2. Differential sensitivity of platelet transduction processes and functional responses to inhibition by cyclic AMP. Biochem J 232: 267–271.

Cao Y, Nishihara R, Wu K, Wang M, Ogino S, Willett WC *et al*. (2016). Population-wide impact of long-term use of aspirin and the risk for cancer. JAMA Oncol 2: 762–769.

Cattaneo M (2007). Resistance to antiplatelet drugs: molecular mechanisms and laboratory detection. J Thromb Haemost 5: 230–237.

Cerletti C, Livio M, De Gaetano G (1982). Non-steroidal anti-inflammatory drugs react with two sites on platelet cyclo-oxygenase. Evidence from "in vivo" drug interaction studies in rats. Biochim Biophys Acta 714: 122–128.

Chan MV, Armstrong PCJ, Papalia F, Kirkby NS, Warner TD (2011). Optical multichannel (optimul) platelet aggregometry in 96-well plates as an additional method of platelet reactivity testing. Platelets 22: 485–494.

Cole BF, Logan RF, Halabi S, Benamouzig R, Sandler RS, Grainge MJ *et al*. (2009). Aspirin for the chemoprevention of colorectal adenomas: meta-analysis of the randomized Trials. J Natl Cancer Inst 101: 256–266.

Collaboration CaTNTC (2013). Vascular and upper gastrointestinal effects of non-steroidal anti-inflammatory drugs: meta-analyses of individual participant data from randomised trials. The Lancet 382: 769–779.

Collet JP, Cuisset T, Range G, Cayla G, Elhadad S, Pouillot C *et al*. (2012). Bedside monitoring to adjust antiplatelet therapy for coronary stenting. N Engl J Med 367: 2100–2109.

Consuegra-Sánchez L, López-Palop R, Cano P, Carrillo P, Picó F, Villegas M *et al*. (2013). Assessment of high on-treatment platelet reactivity in patients with ischemic heart disease: concordance between the Multiplate and VerifyNow assays. J Thromb Haemost 11: 379–381.

Coppinger JA, Cagney G, Toomey S, Kislinger T, Belton O, Mcredmond JP *et al*. (2004). Characterization of the proteins released from activated platelets leads to localization of novel platelet proteins in human atherosclerotic lesions. Blood 103: 2096–2104.

Crescente M, Castelnuovo AD, Iacoviello L, Gaetano GD, Cerletti C (2008a). PFA-100 closure time to predict cardiovascular events in aspirin-treated cardiovascular patients: a meta-analysis of 19 studies comprising 3,003 patients. Thromb Haemost 99: 1129–1131.

Crescente M, Castelnuovo AD, Iacoviello L, Vermylen J, Cerletti C, Gaetano GD (2008b). Response variability to aspirin as assessed by the platelet function analyzer (PFA)-100 – a systematic review. Thromb Haemost 99: 14–26.

Crescente M, Mezzasoma AM, Del Pinto M, Palmerini F, Di Castelnuovo A, Cerletti C *et al*. (2011). Incomplete inhibition of platelet function as assessed by the platelet function analyzer (PFA-100) identifies a subset of cardiovascular patients with high residual platelet response while on aspirin. Platelets 22: 179–187.

Croset M, Sala A, Folco G, Lagarde M (1988). Inhibition by lipoxygenase products of TXA2-like responses of platelets and vascular smooth muscle: 14-Hydroxy from 22:6N-3 is more potent than 12-HETE. Biochem Pharmacol 37: 1275–1280.

Cuzick J, Thorat MA, Bosetti C, Brown PH, Burn J, Cook NR *et al*. (2015). Estimates of benefits and harms of prophylactic use of aspirin in the general population. Ann Oncol 26: 47–57.

Czapiga M, Gao J-L, Kirk A, Lekstrom-Himes J (2005). Human platelets exhibit chemotaxis using functional N-formyl peptide receptors. Exp Hematol 33: 73–84.

Deeb RS, Upmacis RK, Lamon BD, Gross SS, Hajjar DP (2008). Maintaining equilibrium by selective targeting of cyclooxygenase pathways. Promising Offensives Against Vascular Injury. Hypertension 51: 1–7.

den Dekker E, Gorter G, Heemskerk JWM, Akkerman J-WN (2002). Development of platelet inhibition by cAMP during megakaryocytopoiesis. J Biol Chem 277: 29321–29329.

Dennis EA, Norris PC (2015). Eicosanoid storm in infection and inflammation. Nat Rev Immunol 15: 511–523.

Depta JP, Fowler J, Novak E, Katzan I, Bakdash S, Kottke-Marchant K *et al*. (2012). Clinical outcomes using a platelet function-guided approach for secondary prevention in patients with ischemic stroke or transient ischemic attack. Stroke 43: 2376–2381.

Dovizio M, Maier TJ, Alberti S, Di Francesco L, Marcantoni E, Münch G *et al*. (2013). Pharmacological inhibition of platelet-tumor cell cross-talk prevents platelet-induced overexpression of cyclooxygenase-2 in HT29 human colon carcinoma cells. Mol Pharmacol 84: 25–40.

Du G, Lin Q, Wang J (2016). A brief review on the mechanisms of aspirin resistance. Int J Cardiol 220: 21–26.

Etulain J, Fondevila C, Negrotto S, Schattner M (2013). Platelet-mediated angiogenesis is independent of VEGF and fully inhibited by aspirin. Br J Pharmacol 170: 255–265.

Evangelista V, Manarini S, Di Santo A, Capone ML, Ricciotti E, Di Francesco L *et al*. (2006). De novo synthesis of cyclooxygenase-1 counteracts the suppression of platelet thromboxane biosynthesis by aspirin. Circ Res 98: 593–595.

Fabre J-E, Nguyen M, Athirakul K, Coggins K, Mcneish JD, Austin S *et al*. (2001). Activation of the murine EP3 receptor for PGE2 inhibits cAMP production and promotes platelet aggregation. J Clin Invest 107: 603–610.

Ferreira SH, Moncada S, Vane JR (1971). Indomethacin and aspirin abolish prostaglandin release from the spleen. Nat New Biol 231: 237–239.

FitzGerald GA, Oates JA, Hawiger J, Maas RL, Roberts LJ 2nd, Lawson JA *et al*. (1983). Endogenous biosynthesis of prostacyclin and thromboxane and platelet function during chronic administration of aspirin in man. J Clin Invest 71: 676–688.

Franchi F, Rollini F, Aggarwal N, Hu J, Kureti M, Durairaj A *et al*. (2016). Pharmacodynamic comparison of prasugrel versus ticagrelor in patients with type 2 diabetes mellitus and coronary artery disease. Clinical Perspective. *The OPTIMUS (Optimizing Antiplatelet Therapy in Diabetes Mellitus)-4 Study*. Circulation 134: 780–792.

Frelinger AL, Furman MI, Linden MD, Li Y, Fox ML, Barnard MR *et al*. (2006). Residual arachidonic acid–induced platelet activation via an adenosine diphosphate–dependent but cyclooxygenase-1– and cyclooxygenase-2–independent pathway. A 700-Patient Study of Aspirin Resistance. Circulation 113: 2888–2896.

Friedman EA, Ogletree ML, Haddad EV, Boutaud O (2015). Understanding the role of prostaglandin E2 in regulating human platelet activity in health and disease. Thromb Res 136: 493–503.

Gargiulo G, Windecker S, Vranckx P, Gibson CM, Mehran R, Valgimigli M (2016). A critical appraisal of aspirin in secondary prevention: is less more? Circulation 134: 1881–1906.

Gay LJ, Felding-Habermann B (2011). Contribution of platelets to tumour metastasis. Nat Rev Cancer 11: 123–134.

Glenn JR, White AE, Iyu D, Heptinstall S (2012). PGE2 reverses Gs-mediated inhibition of platelet aggregation by interaction with EP3 receptors, but adds to non-Gs-mediated inhibition of platelet aggregation by interaction with EP4 receptors. Platelets 23: 344–351.

Gremmel T, Koppensteiner R, Panzer S (2015). Comparison of aggregometry with flow cytometry for the assessment of agonists-induced platelet reactivity in patients on dual antiplatelet therapy. PLoS One 10: e0129666.

Gross S, Tilly P, Hentsch D, Vonesch J-L, Fabre J-E (2007). Vascular wall-produced prostaglandin E2 exacerbates arterial thrombosis and atherothrombosis through platelet EP3 receptors. J Exp Med 204: 311–320.

Guillem-Llobat P, Dovizio M, Bruno A, Ricciotti E, Cufino V, Sacco A *et al*. (2016). Aspirin prevents colorectal cancer metastasis in mice by splitting the crosstalk between platelets and tumor cells. Oncotarget 7: 32462–32477.

Gurbel PA, Bliden KP, Dichiara J, Newcomer J, Weng W, Neerchal NK *et al*. (2007). Evaluation of dose-related effects of aspirin on platelet function. Results from the Aspirin-Induced Platelet Effect (ASPECT) Study. Circulation 115: 3156–3164.

Harding SD, Sharman JL, Faccenda E, Southan C, Pawson AJ, Ireland S *et al*. (2018). The IUPHAR/BPS Guide to PHARMACOLOGY in 2018: updates and expansion to encompass the new guide to IMMUNOPHARMACOLOGY. Nucl Acids Res 46: D1091–D1106.

Harkewicz R, Dennis EA (2011). Applications of mass spectrometry to lipids and membranes. Annu Rev Biochem 80: 301–325.

Herschman HR, Reddy ST, Xie W (1997). Function and regulation of prostaglandin synthase-2. Adv Exp Med Biol 407: 61–66.

Hla T, Neilson K (1992). Human cyclooxygenase-2 cDNA. Proc Natl Acad Sci U S A 89: 7384–7388.

Investigators TCIUaTPRET (2001). Effects of clopidogrel in addition to aspirin in patients with acute coronary syndromes without ST-segment elevation. N Engl J Med 345: 494–502.

Johnson EN, Brass LF, Funk CD (1998). Increased platelet sensitivity to ADP in mice lacking platelet-type 12-lipoxygenase. Proc Natl Acad Sci U S A 95: 3100–3105.

Kauskot A, Hoylaerts MF (2012). Platelet receptors. In: Gresele P, Born GVR, Patrono C, Page CP (eds). Antiplatelet Agents. Springer Berlin Heidelberg: Berlin, Heidelberg.

Khan KNM, Paulson SK, Verburg KM, Lefkowith JB, Maziasz TJ (2002). Pharmacology of cyclooxygenase-2 inhibition in the kidney. Kidney Int 61: 1210–1219.

Kidson-Gerber G, Weaver J, Gemmell R, Prasan AM, Chong BH (2010). Serum thromboxane B2 compared to five other platelet function tests for the evaluation of aspirin effect in stable cardiovascular Disease. Heart Lung Circ 19: 234–242.

Kirkby NS, Leadbeater PDM, Chan MV, Nylander S, Mitchell JA, Warner TD (2011). Antiplatelet effects of aspirin vary with level of P2Y12 receptor blockade supplied by either ticagrelor or prasugrel. J Thromb Haemost 9: 2103–2105.

Kirkby NS, Lundberg MH, Harrington LS, Leadbeater PDM, Milne GL, Potter CMF *et al*. (2012). Cyclooxygenase-1, not cyclooxygenase-2, is responsible for physiological production of prostacyclin in the cardiovascular system. Proc Natl Acad Sci U S A 109: 17597–17602.

Kirkby NS, Reed DM, Edin ML, Rauzi F, Mataragka S, Vojnovic I *et al*. (2015). Inherited human group IVA cytosolic phospholipase A2 deficiency abolishes platelet, endothelial, and leucocyte eicosanoid generation. FASEB J 29: 4568–4578.

Krasopoulos G, Brister SJ, Beattie WS, Buchanan MR (2008). Aspirin "resistance" and risk of cardiovascular morbidity: systematic review and meta-analysis. BMJ 336: 195–198.

Kujubu DA, Fletcher BS, Varnum BC, Lim RW, Herschman HR (1991). TIS10, a phorbol ester tumor promoter-inducible mRNA from Swiss 3T3 cells, encodes a novel prostaglandin synthase/cyclooxygenase homologue. J Biol Chem 266: 12866–12872.

Kune GA, Kune S, Watson LF (1988). Colorectal cancer risk, chronic illnesses, operations, and medications: case control results from the Melbourne Colorectal Cancer Study. Cancer Res 48: 4399–4404.

Langenbach R, Morham SG, Tiano HF, Loftin CD, Ghanayem BI, Chulada PC *et al*. (1997). Disruption of the mouse cyclooxygenase 1 gene. Characteristics of the mutant and areas of future study. Adv Exp Med Biol 407: 87–92.

Li J, Jian Z, Song M, Guo W, Chen G, Lu W*et al*. (2014). Tailored antiplatelet therapy and clinical adverse outcomes. Heart 100: 41–46.

Loll PJ, Picot D, Garavito RM (1995). The structural basis of aspirin activity inferred from the crystal structure of inactivated prostaglandin H2 synthase. Nat Struct Biol 2: 637–643.

Lordkipanidzé M, Lowe GC, Kirkby NS, Chan MV, Lundberg MH, Morgan NV *et al*. (2014). Characterization of multiple platelet activation pathways in patients with bleeding as a high-throughput screening option: use of 96-well Optimul assay. Blood 123: e11–e22.

Lordkipanidzé M, Pharand C, Schampaert E, Turgeon J, Palisaitis DA, Diodati JG (2007). A comparison of six major platelet function tests to determine the prevalence of aspirin resistance in patients with stable coronary artery disease. Eur Heart J 28: 1702–1708.

Mahaffey KW, Wojdyla DM, Carroll K, Becker RC, Storey RF, Angiolillo DJ *et al*. (2011). Ticagrelor compared with clopidogrel by geographic region in the platelet inhibition and patient outcomes (PLATO) Trial. Clinical Perspective. Circulation 124: 544–554.

Marcus AJ, Weksler BB, Jaffe EA, Broekman MJ (1980). Synthesis of prostacyclin from platelet-derived endoperoxides by cultured human endothelial cells. J Clin Invest 66: 979–986.

Maree AO, Fitzgerald DJ (2007). Variable platelet response to aspirin and clopidogrel in atherothrombotic disease. Circulation 115: 2196–2207.

Margalit A, Gilutz H, Granot Y (1995). Original article: low regulatory volume decrease rate in platelets from ischemic patients: a possible role for hepoxilin A3 in thrombogenicity. Platelets 6: 371–376.

Masferrer JL, Seibert K, Zweifel B, Needleman P (1992). Endogenous glucocorticoids regulate an inducible cyclooxygenase enzyme. Proc Natl Acad Sci U S A 89: 3917–3921.

Maskrey BH, Rushworth GF, Law MH, Treweeke AT, Wei J, Leslie SJ *et al*. (2014). 12-Hydroxyeicosatetraenoic acid is associated with variability in aspirin-induced platelet inhibition. J Inflamm 11: 33.

Mehta P, Mehta J, Lawson D, Krop I, Letts LG (1986). Leukotrienes potentiate the effects of epinephrine and thrombin on human platelet aggregation. Thromb Res 41: 731–738.

Midgett C, Stitham J, Martin KA, Hwa J (2011). Prostacyclin receptor regulation – from transcription to trafficking. Curr Mol Med 11: 517–527.

Mitchell JA, Warner TD (2006). COX isoforms in the cardiovascular system: understanding the activities of non-steroidal anti-inflammatory drugs. Nat Rev Drug Discov 5: 75–86.

Mitchell JA, Kirkby NS (2018). Eicosanoids, prostacyclin and cyclooxygenase in the cardiovascular system. Br J Pharmacology. [Epub ahead of print]

Moncada S, Gryglewski R, Bunting S, Vane JR (1976). An enzyme isolated from arteries transforms prostaglandin endoperoxides to an unstable substance that inhibits platelet aggregation. Nature 263: 663–665.

Moncada S, Korbut R, Bunting S, Vane JR (1978). Prostacyclin is a circulating hormone. Nature 273: 767–768.

Needleman P, Moncada S, Bunting S, Vane JR, Hamberg M, Samuelsson B (1976). Identification of an enzyme in platelet microsomes which generates thromboxane A2 from prostaglandin endoperoxides. Nature 261: 558–560.

O'Banion MK, Winn VD, Young DA (1992). cDNA cloning and functional activity of a glucocorticoid-regulated inflammatory cyclooxygenase. Proc Natl Acad Sci U S A 89: 4888–4892.

Oelz O, Oelz R, Knapp HR, Sweetman BJ, Oates JA (1977). Biosynthesis of prostaglandin D2. 1. Formation of prostaglandin D2 by human platelets. Prostaglandins 13: 225–234.

Ohmori T, Yatomi Y, Nonaka T, Kobayashi Y, Madoiwa S, Mimuro J *et al*. (2006). Aspirin resistance detected with aggregometry cannot be explained by cyclooxygenase activity: involvement of other signaling pathway(s) in cardiovascular events of aspirin-treated patients. J Thromb Haemost 4: 1271–1278.

Ortiz-Muñoz G, Mallavia B, Bins A, Headley M, Krummel MF, Looney MR (2014). Aspirin-triggered 15-epi-lipoxin A4 regulates neutrophil-platelet aggregation and attenuates acute lung injury in mice. Blood 124: 2625–2634.

Patrignani P, Filabozzi P, Patrono C (1982). Selective cumulative inhibition of platelet thromboxane production by low-dose aspirin in healthy subjects. J Clin Invest 69: 1366–1372.

Patrignani P, Patrono C (2016). Aspirin and cancer. J Am Coll Cardiol 68: 967–976.

Patrono C (2005). Low-dose aspirin for the prevention of atherothrombosis. N Engl J Med 353: 2373–2383.

Patrono C, Andreotti F, Arnesen H, Badimon L, Baigent C, Collet JP *et al*. (2011). Antiplatelet agents for the treatment and prevention of atherothrombosis. Eur Heart J 32: 2922–2932.

Petrucci G, De Cristofaro R, Rutella S, Ranelletti FO, Pocaterra D, Lancellotti S *et al*. (2011). Prostaglandin E2 differentially modulates human platelet function through the prostanoid EP2 and EP3 receptors. J Pharmacol Exp Ther 336: 391–402.

Piper PJ, Vane JR (1969). Release of additional factors in anaphylaxis and its antagonism by anti-inflammatory drugs. Nature 223: 29–35.

Porro B, Songia P, Squellerio I, Tremoli E, Cavalca V (2014). Analysis, physiological and clinical significance of 12-HETE: a neglected platelet-derived 12-lipoxygenase product. J Chromatogr B 964: 26–40.

Rauzi F, Kirkby NS, Edin ML, Whiteford J, Zeldin DC, Mitchell JA *et al*. (2016). Aspirin inhibits the production of proangiogenic 15(S)-HETE by platelet cyclooxygenase-1. FASEB J 30: 4256–4266.

Reilly M, Fitzgerald GA (1993). Cellular activation by thromboxane A2 and other eicosanoids. Eur Heart J 14 (Suppl K): 88–93.

Reny JL, De Moerloose P, Dauzat M, Fontana P (2008). Use of the PFA-100™ closure time to predict cardiovascular events in aspirin-treated cardiovascular patients: a systematic review and meta-analysis. J Thromb Haemost 6: 444–450.

Reynaud D (2002). The hepoxilin analog PBT-3 inhibits heparin-activated platelet aggregation evoked by ADP. FEBS Lett 515: 58–60.

Rocca B, Secchiero P, Ciabattoni G, Ranelletti FO, Catani L, Guidotti L *et al*. (2002). Cyclooxygenase-2 expression is induced during human megakaryopoiesis and characterizes newly formed platelets. Proc Natl Acad Sci U S A 99: 7634–7639.

Rothwell PM, Fowkes FGR, Belch JFF, Ogawa H, Warlow CP, Meade TW (2011). Effect of daily aspirin on long-term risk of death due to cancer: analysis of individual patient data from randomised trials. The Lancet 377: 31–41.

Rothwell PM, Price JF, Fowkes FGR, Zanchetti A, Roncaglioni MC, Tognoni G *et al*. (2012). Short-term effects of daily aspirin on cancer incidence, mortality, and non-vascular death: analysis of the time course of risks and benefits in 51 randomised controlled trials. The Lancet 379: 1602–1612.

Santilli F, Rocca B, De Cristofaro R, Lattanzio S, Pietrangelo L, Habib A *et al*. (2009). Platelet cyclooxygenase inhibition by low-dose aspirin is not reflected consistently by platelet function assays: implications for aspirin "resistance". J Am Coll Cardiol 53: 667–677.

Smith JB, Willis AL (1971). Aspirin selectively inhibits prostaglandin production in human platelets. Nat New Biol 231: 235–237.

Smith JP, Haddad EV, Taylor MB, Oram D, Blakemore D, Chen Q *et al*. (2012). Suboptimal inhibition of platelet cyclooxygenase-1 by aspirin in metabolic syndrome. Hypertension 59: 719–725.

Smith WL, Dewitt DL (1996). Prostaglandin endoperoxide H synthases-1 and -2. Adv Immunol 62: 167–215.

Smyth EM (2010). Thromboxane and the thromboxane receptor in cardiovascular disease. Clin Lipidol 5: 209–219.

Snoep JD, Hovens MC, Eikenboom JJ, Van Der Bom JG, Huisman MV (2007). Association of laboratory-defined aspirin resistance with a higher risk of recurrent cardiovascular events: A systematic review and meta-analysis. Arch Intern Med 167: 1593–1599.

Steinbach G, Lynch PM, Phillips RKS, Wallace MH, Hawk E, Gordon GB *et al*. (2000). The effect of celecoxib, a cyclooxygenase-2 inhibitor, in familial adenomatous polyposis. N Engl J Med 342: 1946–1952.

Subhash PK, David SG, David RJ, Letts LG (2007). Eicosanoids in Inflammation: biosynthesis, pharmacology, and therapeutic frontiers. Curr Top Med Chem 7: 311–340.

Sudhahar V, Shaw S, Imig JD (2010). Epoxyeicosatrienoic acid analogs and vascular function. Curr Med Chem 17: 1181–1190.

Svensson J, Hamberg M, Samuelsson B (1975). Prostaglandin endoperoxides IX. Characterization of rabbit aorta contracting substance (RCS) from guinea pig lung and human platelets. Acta Physiol Scand 94: 222–228.

Tantry US, Bonello L, Aradi D, Price MJ, Jeong Y-H, Angiolillo DJ *et al*. (2013). Consensus and update on the definition of on-treatment platelet reactivity to adenosine diphosphate associated with ischemia and bleeding. J Am Coll Cardiol 62: 2261–2273.

Thiagarjan P, Wu KK (2002). In vitro assays for evaluating platelet function. In: Gresele P, Page C, Fuster V, Vermylyn J (eds). Platelets in thrombotic and non thrombotic disorders. Cambridge University Press: Cambridge, UK.

Ulrych T, Böhm A, Polzin A, Daum G, Nüsing RM, Geisslinger G *et al*. (2011). Release of sphingosine-1-phosphate from human platelets is dependent on thromboxane formation. J Thromb Haemost 9: 790–798.

Vane JR (1971). Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. Nat New Biol 231: 232–235.

Wallace JL, Devchand PR (2005). Emerging roles for cyclooxygenase-2 in gastrointestinal mucosal defense. Br J Pharmacol 145: 275–282.

Wallentin L, Becker RC, Budaj A, Cannon CP, Emanuelsson H, Held C *et al*. (2009). Ticagrelor versus clopidogrel in patients with acute coronary syndromes. N Engl J Med 361: 1045–1057.

Warner TD, Armstrong PC, Chan MV, Knowles RB (2016). The importance of endothelium-derived mediators to the efficacy of dual anti-platelet therapy. Expert Rev Hematol 9: 223–225.

Warner TD, Armstrong PCJ, Curzen NP, Mitchell JA (2010). Dual antiplatelet therapy in cardiovascular disease: does aspirin increase clinical risk in the presence of potent P2Y12 receptor antagonists? Heart 96: 1693–1694.

Warner TD, Nylander S, Whatling C (2011). Anti-platelet therapy: cyclo-oxygenase inhibition and the use of aspirin with particular regard to dual anti-platelet therapy. Br J Clin Pharmacol 72: 619–633.

Whittaker N, Bunting S, Salmon J, Moncada S, Vane JR, Johnson RA *et al*. (1976). The chemical structure of prostaglandin X (prostacyclin). Prostaglandins 12: 915–928.

Whittle BJ, Moncada S, Vane JR (1978). Comparison of the effects of prostacyclin (PGI2), prostaglandin E1 and D2 on platelet aggregation in different species. Prostaglandins 16: 373–388.

Whittle BJ, Silverstein AM, Mottola DM, Clapp LH (2012). Binding and activity of the prostacyclin receptor (IP) agonists, treprostinil and iloprost, at human prostanoid receptors: treprostinil is a potent DP1 and EP2 agonist. Biochem Pharmacol 84: 68–75.

Windecker S, Kolh P, Alfonso F, Collet J-P, Cremer J, Falk V *et al*. (2014). 2014 ESC/EACTS Guidelines on myocardial revascularization The Task Force on Myocardial Revascularization of the European Society of Cardiology (ESC) and the European Association for Cardio-Thoracic Surgery (EACTS)Developed with the special contribution of the European Association of Percutaneous Cardiovascular Interventions (EAPCI). Eur Heart J 35: 2541–2619.

Wiviott SD, Braunwald E, Mccabe CH, Montalescot G, Ruzyllo W, Gottlieb S *et al*. (2007). Prasugrel versus clopidogrel in patients with acute coronary syndromes. N Engl J Med 357: 2001–2015.

Xie WL, Chipman JG, Robertson DL, Erikson RL, Simmons DL (1991). Expression of a mitogen-responsive gene encoding prostaglandin synthase is regulated by mRNA splicing. Proc Natl Acad Sci U S A 88: 2692–2696.

Yang J, Wu J, Jiang H, Mortensen R, Austin S, Manning DR *et al*. (2002). Signaling through Gi family members in platelets: redundancy and specificity in the regulation of adenylyl cyclase and other effectors. J Biol Chem 277: 46035–46042.

Yang L (2015). The role of epoxyeicosatrienoic acids in the cardiovascular system. Br J Clin Pharmacol 80: 28–44.

Yu Y, Ricciotti E, Scalia R, Tang SY, Grant G, Yu Z *et al*. (2012). Vascular COX-2 modulates blood pressure and thrombosis in mice. Sci Transl Med 4: 132ra54–132ra54.